

Amendments to the claims:

This listing of the claims will replace all prior versions, and listings, of claims in the application.

1-28. (cancelled).

29. (currently amended) An ~~in-vivo~~ assay to identify molecular markers linked to phenotypic stability of a chondrocyte cell population comprising:

a) providing a suspension of isolated or expanded cells and determining markers thereof.

~~a) b)~~ injecting intramuscularly or subcutaneously in a non-human animal a said suspension of isolated or expanded cells in an iso-osmotic liquid, the same suspension comprising articular chondrocytes in an amount equivalent to at least  $1 \times 10^6$  chondrocytes as applied to immune-deficient mice,

~~b) c)~~ allowing the formation of cartilaginous tissue in vivo,

~~c) d)~~ sacrificing the animal,

~~d) e)~~ evaluating the in vivo formed cartilage histologically for stable, non-vascularised cartilage ~~in-vivo~~, and

~~e) f)~~ identifying positive or negative molecular markers of those isolated or expanded cells ~~evaluated in step d)~~ which formed stable, non-vascularised cartilage in vivo, as evaluated in step e).

30. (currently amended) An assay to identify molecular markers according to claim 29, comprising using freshly isolated or serially passaged cells and using differential gene expression analysis methods ~~including~~ selected from the group consisting of differential display, subtractive hybridization, subtracted libraries or cDNA chips and cDNA arrays to identify said positive or negative molecular markers of those isolated or expanded cells which formed stable, non-vascularized cartilage in vivo.

31. (currently amended) A method to identify cells having chondrocyte phenotypic stability comprising determining the expression of BMP-2 and/or FGFR-3 and/or markers co-detectable with these markers identified by the method of claim 29 and/or specific reporter constructs ~~associated with these~~ comprising a promoter of said markers.

32. (currently amended) A method to identify cells having chondrocyte phenotypic stability according to claim 31 further comprising determining that activin-like kinase-1 (ALK-1) is not expressed, and/or markers co-detectable with these markers identified by the method of claim 29 and/or specific reporter constructs comprising a promoter of said ~~associated with these~~ markers are not expressed.

33. (currently amended) A method to identify cells having chondrocyte phenotypic stability comprising

a) hybridizing sets of DNA probes to messenger RNA from cells, sets of DNA probes provided on DNA arrays or DNA chips, said DNA probes being probes of positive and negative markers for chondrocyte phenotypic stability; and

b) identifying cells that (i) hybridise with said positive markers and (ii) do not hybridise with said negative markers for chondrocyte phenotypic stability.

34. (currently amended) A method to identify phenotypically stable primary chondrocytes and chondrocytes, after at least one passage, that remained phenotypically stable comprising detecting sets of positive markers, said positive markers being selected from expressed BMP-2, FGFR-3, markers co-detectable with these markers identified by the method of claim 29 or specific reporter constructs comprising a promoter of said ~~associated with these markers or markers determined by an in vivo assay according to~~ claim 29.

35. (currently amended) Method to monitor passage by passage cell expansion and/or to predict when cell expansion must be stopped and/or to recover cells that have already lost their phenotypic stability only when needed and/or to provide a means for quality control of cells to be used for autologous cell transplantation and/or selecting from a cell population only those cells that retain their chondrocyte phenotypic stability, comprising detecting the expression of molecular markers of chondrocyte phenotypic stability selected from the group of:

-(i) markers determined by the assay comprising:

- a) injecting intramuscularly or subcutaneously in a non-human animal a suspension of isolated or expanded cells in an iso-osmotic liquid, the same suspension comprising articular chondrocytes in an amount equivalent to at least  $1 \times 10^6$  chondrocytes as applied to immune-deficient mice,
- b) allowing the formation of cartilaginous tissue,
- c) sacrificing the animal,
- d) ~~evaluate~~ evaluating the formed cartilage histologically for stable, non-vascularised cartilage in vivo, and
- e) ~~identify~~ identifying positive or negative molecular markers of those isolated or expanded cells evaluated in step d) which form stable, non-vascularised cartilage in vivo.

-(ii) BMP-2 and/or FGFR-3 and/or markers co-detectable with these markers identified by the method of claim 29 and/or specific reporter constructs associated with these markers, and

-(iii) expressed activin-like kinase-1 (ALK-1) as a marker negatively associated with chondrocyte phenotypic stability, and/or markers co-detectable with these markers identified by the method of claim 29 and/or specific reporter constructs comprising a promoter of said ~~associated with these markers.~~

36. (previously presented) Method according to claim 35, comprising sorting cells via monoclonal or polyclonal antibodies against negative or positive markers or co detectable markers for the monitoring of cell expansion and/or predicting when cell expansion must be stopped and/or selecting cells which have lost chondrocyte phenotypic stability and/or selecting cells which retain chondrocyte phenotypic stability.

37 – 38. (cancelled)

39. (currently amended) Cells identified according to ~~claim 33~~ a method comprising:

(a) hybridising sets of DNA probes to messenger RNA from cells, said DNA probes being probes of positive and negative markers for chondrocyte phenotypic stability; and

(b) identifying those cells that (i) hybridise with said positive markers and (ii) do not hybridise with said negative markers for chondrocyte phenotypic stability.

40. (currently amended) Cells selected according to ~~claim 35~~ a method comprising

(a) detecting the expression of molecular markers of chondrocyte phenotypic stability selected from the group consisting of:

(i) BMP-2 and/or FGFR-3 and/or markers co-detectable with these markers identified by the method of claim 29 and/or specific reporter constructs associated with these markers, which markers are positive markers for chondrocyte phenotypic stability, and

(ii) expressed activin-like kinase-1 (ALK-1) as a marker negatively associated with chondrocyte phenotypic stability, and/or markers co-detectable with these markers identified by the method of claim 29 and/or specific reporter constructs comprising a said marker; and

(b) selecting cells in which expression of positive markers and absence of markers negatively associated with phenotypic stability is detected.

41. (currently amended) An ~~in-vivo~~ assay to predict the outcome of autologous cell transplantation comprising ~~to predict the outcome of autologous cell transplantation using the cells identified by the method of claim 33~~

~~or to optimize cell culture conditions and manufacturing processes for a specific application in tissue engineering of cells identified by the method of claim 33, said selected cells being used for autologous cell transplantation or being used in a pharmaceutical composition, that a treatment administered to the selected cells can hamper or enhance the anchorage independent growth of said population as well as its phenotypic stability;~~

~~the in vivo assay comprising:~~

(a) providing isolated or expanded chondrocytes;

(b) ~~subcutaneous or intramuscular injection~~ subcutaneously or intramuscularly injecting in a non-human animal of a suspension of the selected cells in an iso-osmotic liquid, the same said suspension comprising articular chondrocytes in an amount equivalent to at least  $1 \times 10^6$  chondrocytes as applied to immune-deficient mice;

(c) allowing the formation of cartilaginous tissue;

(d) sacrificing the animal; and

(e) evaluating the in vivo formed cartilage histologically for stable, non-vascularised cartilage; whereby the formation of stable, non-vascularised cartilage is indicative of a positive outcome of autologous cell transplantation using said isolated or expanded chondrocytes.

42. (cancelled)

43. (currently amended) ~~Transplanting~~ A method of transplanting cells to a connective tissue site in a patient or a method of seeding with cells any prosthetic device intended to be anchored into a mammal, wherein said cells are cells which retaining retain their chondrocyte phenotypic stability and which are identified according to the method of any one of claims 31 to 33.

44. (currently amended) ~~Transplanting~~ A method of transplanting cells to a connective tissue site in a patient or a method of seeding with cells any prosthetic device intended to be anchored into a mammal, wherein said cells are cells which retaining retain their chondrocyte phenotypic stability and which are selected according to the method of claims 35.

45. (previously presented) A therapeutic composition for humans including cells identified according to claim any one of claims 31 to 33, optionally further including at least a pharmaceutically acceptable carrier and/or a growth factor.

46. (previously presented) A therapeutic composition for humans including cells selected according to claim 35, optionally further including at least a pharmaceutically acceptable carrier and/or a growth factor.

47. (currently amended) A diagnostic comprising ~~DNA chips of claim 33~~ positive or negative markers identified according to the method of claim 29.

48. (cancelled)

49. (currently amended) A cell culture exhibiting chondrocyte phenotypic stability in which the cells express a ratio of BMP-2 and/or FGFR-3 as molecular markers positively associated with chondrocyte phenotypic stability and/or markers ~~co-detectable~~

co-detectable with these markers identified by the method of claim 29 and/or specific reporter constructs comprising associated with these markers to activin-like kinase-1 (ALK-1) as a molecular marker negatively associated with chondrocyte phenotypic stability and/or markers co-detectable with this marker identified by the method of claim 29 and/or specific reporter constructs comprising a promoter of said associated with this negative marker, which is greater than 1, ~~preferably greater than 2.~~

50. (currently amended) A cell culture exhibiting chondrocyte phenotypic stability in ~~which the cells~~ which culture does not express activin-like kinase-1 (ALK-1) and/or markers co-detectable with this marker identified by the method of claim 29 and/or specific reporter constructs comprising a promoter of said associated with these markers as ~~molecular markers negatively associated with chondrocyte phenotypic stability.~~

51. (new) The method of claim 33, wherein said DNA probes are provided on a DNA array or a DNA chip.

52. (new) The method of claim 39, wherein said DNA probes are provided on a DNA array or a DNA chip.

53. (new) The diagnostic of claim 47, wherein said positive or negative markers are DNA probes.

54. (new) The cell culture exhibiting chondrocyte phenotypic stability, according to claim 49, wherein said ratio is greater than 2.

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